

# Supporting Information

## Ingestion and chronic effects of car tire tread particles on freshwater benthic macroinvertebrates

*Paula E. Redondo-Hasselerharm<sup>†\*</sup>, Vera N. de Ruijter<sup>‡</sup>, Svenja M. Mintenig<sup>‡§</sup>, Anja Verschoor<sup>‡</sup>, Albert A. Koelmans<sup>†</sup>*

<sup>†</sup>Aquatic Ecology and Water Quality Management group, Wageningen University & Research, P.O. Box 47, 6700 AA Wageningen, The Netherlands

<sup>‡</sup>Copernicus Institute of Sustainable Development, Utrecht University, 3584 CS Utrecht, The Netherlands

<sup>§</sup>KWR Watercycle Research Institute, 3433 PE Nieuwegein, The Netherlands

<sup>‡</sup>National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands

19 pages

7 tables

8 figures

\*Corresponding author: [paula.redondohasselerharm@wur.nl](mailto:paula.redondohasselerharm@wur.nl)

**Table S1. Car tires used for the manufacturing of TP.**

| <b>Nr</b> | <b>Brand</b> | <b>Tire</b>   | <b>Type<sup>a</sup></b> | <b>DOT-code<sup>b</sup></b> | <b>Sidewall-markings</b> |
|-----------|--------------|---------------|-------------------------|-----------------------------|--------------------------|
| 1         | Goodyear     | Ultragrip 7   | w                       | H30F 2FOR                   | 205/55 R16 91 T          |
| 2         | Michelin     | Energy Radial | s                       | DU8X 2201                   | 175/65 R14 82 T          |
| 3         | Dunlop       | SP Sport 2000 | s                       | K5 5F 12W                   | 195/55 R15 85 V          |
| 4         | Dunlop       | SP Sport 07   | s                       | N5HR JC1R                   | 175/70 R14 84 T          |
| 5         | Continental  | ContiEco      | s                       | CNU4 PVB9                   | 195/50 R15 82 T          |

<sup>a</sup> w=winter tire s=summer tire

<sup>b</sup> DOT=Department of transportation; DOT-codes provide information on production date and location.

**Table S2: TGA, GC-MS and ICP-MS instrumental settings to analyse car tire TP.**

|                |                            |   |
|----------------|----------------------------|---|
| <b>TGA</b>     | <b>Instrument</b>          | TGA/DSC 3+, Mettler Toledo  |
|                | <b>Temperature program</b> | 30 to 850 °C, at a constant heating rate of 20 °C   |
|                | <b>Purge gas</b>           | 30 to 600°C: Nitrogen (50 ml min <sup>-1</sup> )<br>600 to 850 °C: Air (50 ml min <sup>-1</sup> ) |
|                | <b>Sample mass</b>         | Approximately 3 mg  |
|                | <b>Sample holder (cup)</b> | Aluminium oxide, 70 µl  |
| <b>GC</b>      | <b>Instrument</b>          | GC (7820A), Agilent Technologies  |
|                | <b>Sample injection</b>    | Manual, 2 µl  |
|                | <b>Injector</b>            | Split- splitless  |
|                | <b>Mode</b>                | Split ratio 7:1   |
|                | <b>Temperature</b>         | 250   |
|                | <b>Flow</b>                | 1 ml min <sup>-1</sup>  |
|                | <b>Temperature program</b> | 50 → 300 at 8.5 degrees min <sup>-1</sup>   |
| <b>MS</b>      | <b>Instrument</b>          | MS (5977B), Agilent Technologies  |
|                | <b>Ionization energy</b>   | 70 eV   |
|                | <b>Scan rate</b>           | 8.6 scans sec <sup>-1</sup>   |
|                | <b>Mass range</b>          | 35- 330   |
| <b>ICP- MS</b> | <b>Instrument</b>          | X Series 2, Thermo Fisher Scientific  |
|                | <b>Forwarded Power</b>     | 1200 W  |
|                | <b>Nebuliser Gas Flow</b>  | 0.9 L min <sup>-1</sup>   |
|                | <b>Auxiliary Gas Flow</b>  | 0.8 L min <sup>-1</sup>   |
|                | <b>Cool Gas Flow</b>       | 13.0 L min <sup>-1</sup>  |
|                | <b>CCT Gas</b>             | He/H Mixture (with 7% H)  |
|                | <b>CCT Gas Flow</b>        | 5.2 mL min <sup>-1</sup>  |
|                | <b>Dwell Time</b>          | 100 ms  |

**Table S3: Polymer pyrolysates detected in the used car tire tread particles by TGA coupled to GC-MS.**

| Original polymer type <sup>(a)</sup> | Pyrolysate <sup>(b)</sup>    | Target ion (qualifiers) | Present retention time <sup>(c)</sup> |
|--------------------------------------|------------------------------|-------------------------|---------------------------------------|
| Polyisoprene                         | Methyl-butadiene (monomer)   | 67 (68,53,39)           | 6.8 min                               |
|                                      | Dipentene (dimer)            | 68 (93, 136)            | 6.8 min                               |
| Styrene butadiene rubber (SBR)       | Butadiene (monomer)          | 39 (54, 53)             | 4.1 min                               |
|                                      | Styrene (monomer)            | 104 (103, 78, 51)       | 4.3 min                               |
| Benzothiazole (vulcanisator)         | Benzothiazole (vulcanisator) | 135 (108)               | 10.9 min                              |

**(a)** *Polymers used during tire production*<sup>1</sup>.

**(b)** *Pyrolysates of the polymers used during tire production (i.e. indicated under (a),<sup>1</sup>, identified in the present study.*

**(c)** *GC-MS retention times assessed in the present study, see also Fig S4.*

**Table S4. Mean ( $\pm$ SD) temperature ( $^{\circ}$ C), pH, DO (%) and EC ( $\mu$ S/cm) in each treatment for all species tested.**

All parameters were measured in the 3 replicates at 5 time points along the experiment, except for EC, which was only measured at the start and at the end of the experiment.

| <i>G. pulex</i>      | 0%               | 0.1 %             | 0.3 %             | 1%                | 3%                | 10%               |
|----------------------|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| T ( $^{\circ}$ C)    | 15.6 $\pm$ 0.06  | 15.6 $\pm$ 0.05   | 15.6 $\pm$ 0.07   | 15.6 $\pm$ 0.05   | 15.6 $\pm$ 0.06   | 15.6 $\pm$ 0.04   |
| pH                   | 8.14 $\pm$ 0.08  | 8.15 $\pm$ 0.13   | 8.15 $\pm$ 0.12   | 8.24 $\pm$ 0.08   | 8.23 $\pm$ 0.08   | 8.43 $\pm$ 0.1    |
| DO (%)               | 96.2 $\pm$ 2.67  | 96.1 $\pm$ 2.70   | 97.2 $\pm$ 1.04   | 97.6 $\pm$ 1.29   | 96.1 $\pm$ 1.44   | 96.7 $\pm$ 0.71   |
| EC ( $\mu$ S/cm)     | 694.8 $\pm$ 79.9 | 700.7 $\pm$ 91.6  | 677.8 $\pm$ 144.6 | 744.7 $\pm$ 114.9 | 762.8 $\pm$ 135.4 | 623.3 $\pm$ 78.9  |
| <i>A. aquaticus</i>  | 0%               | 0.1 %             | 0.3 %             | 1%                | 3%                | 10%               |
| T ( $^{\circ}$ C)    | 15.6 $\pm$ 0.04  | 15.6 $\pm$ 0.04   | 15.6 $\pm$ 0.03   | 15.6 $\pm$ 0.06   | 15.6 $\pm$ 0.06   | 15.6 $\pm$ 0.03   |
| pH                   | 8.14 $\pm$ 0.07  | 8.13 $\pm$ 0.14   | 8.14 $\pm$ 0.08   | 8.23 $\pm$ 0.07   | 8.29 $\pm$ 0.08   | 8.3 $\pm$ 0.1     |
| DO (mg/L)            | 96.9 $\pm$ 0.77  | 96.5 $\pm$ 1.25   | 96.6 $\pm$ 0.92   | 96.4 $\pm$ 1.71   | 97.1 $\pm$ 1.07   | 93.6 $\pm$ 7.78   |
| EC ( $\mu$ S/cm)     | 640.0 $\pm$ 62.4 | 782.3 $\pm$ 152.0 | 615.2 $\pm$ 42.2  | 851.7 $\pm$ 162.0 | 700.8 $\pm$ 70.1  | 724.7 $\pm$ 139.4 |
| <i>Tubifex</i> spp.  | 0%               | 0.1 %             | 0.3 %             | 1%                | 3%                | 10%               |
| T ( $^{\circ}$ C)    | 15.6 $\pm$ 0.09  | 15.6 $\pm$ 0.09   | 15.7 $\pm$ 0.04   | 15.6 $\pm$ 0.06   | 15.6 $\pm$ 0.06   | 15.6 $\pm$ 0.05   |
| pH                   | 8.13 $\pm$ 0.14  | 8.10 $\pm$ 0.16   | 8.10 $\pm$ 0.16   | 8.17 $\pm$ 0.14   | 8.31 $\pm$ 0.08   | 8.42 $\pm$ 0.14   |
| DO (mg/L)            | 96.5 $\pm$ 2.6   | 97.0 $\pm$ 0.83   | 97.0 $\pm$ 0.93   | 93.8 $\pm$ 9.14   | 96.3 $\pm$ 1.49   | 96.9 $\pm$ 0.96   |
| EC ( $\mu$ S/cm)     | 669.8 $\pm$ 72.0 | 639.3 $\pm$ 50.3  | 622.8 $\pm$ 48.2  | 596.7 $\pm$ 41.9  | 624.0 $\pm$ 64.0  | 674.8 $\pm$ 82.8  |
| <i>L. variegatus</i> | 0%               | 0.1 %             | 0.3 %             | 1%                | 3%                | 10%               |
| T ( $^{\circ}$ C)    | 15.6 $\pm$ 0.09  | 15.7 $\pm$ 0.31   | 15.6 $\pm$ 0.04   | 15.6 $\pm$ 0.06   | 15.6 $\pm$ 0.08   | 15.6 $\pm$ 0.05   |
| pH                   | 8.20 $\pm$ 0.10  | 8.12 $\pm$ 0.08   | 8.20 $\pm$ 0.12   | 8.19 $\pm$ 0.09   | 8.29 $\pm$ 0.13   | 8.45 $\pm$ 0.12   |
| DO (mg/L)            | 97.0 $\pm$ 0.84  | 96.2 $\pm$ 1.23   | 96.6 $\pm$ 1.59   | 97.5 $\pm$ 0.81   | 96.1 $\pm$ 3.00   | 96.6 $\pm$ 1.23   |
| EC ( $\mu$ S/cm)     | 674.0 $\pm$ 91.4 | 607.7 $\pm$ 34.9  | 657.7 $\pm$ 84.3  | 612.3 $\pm$ 44.7  | 639.7 $\pm$ 65.2  | 640.1 $\pm$ 80.0  |

**Table S5. Measurements of Zn, S, Cd, Cr, Cu, Ni and Pb concentrations at six car tire TP concentrations in sediment DW (0, 0.1, 0.3, 1.0, 3.0 and 10 %) using:**

**A) Extraction with HNO<sub>3</sub>-HCL.**

| Treatment       | Zn<br>[mg/kg] | S<br>[mg/kg] | Cd<br>[mg/kg] | Cr<br>[mg/kg] | Cu<br>[mg/kg] | Ni<br>[mg/kg] | Pb<br>[mg/kg] |
|-----------------|---------------|--------------|---------------|---------------|---------------|---------------|---------------|
| Detection limit | 5             | 30           | 0.05          | 0.8           | 3             | 1.6           | 0.3           |
| 0%              | 75            | 13711        | 0.47          | 47.5          | 26            | 31.7          | 31.9          |
| 0.10%           | 84            | 14441        | 0.53          | 49.1          | 26            | 33.1          | 33.4          |
| 0.30%           | 143           | 14267        | 0.49          | 46.8          | 25            | 31.3          | 32.1          |
| 1.0%            | 97            | 14292        | 0.53          | 47.5          | 25            | 31.8          | 33.0          |
| 3.0%            | 277           | 14086        | 0.52          | 47.0          | 26            | 31.9          | 32.5          |
| 10%             | 735           | 13253        | 0.54          | 42.1          | 24            | 27.7          | 29.1          |

**B) Extraction with 0.01M CaCl<sub>2</sub>.**

| Treatment       | Zn<br>[mg/kg] | S<br>[mg/kg] | Cd<br>[µg/kg] | Cr<br>[µg/kg] | Cu<br>[µg/kg] | Ni<br>[µg/kg] | Pb<br>[µg/kg] |
|-----------------|---------------|--------------|---------------|---------------|---------------|---------------|---------------|
| Detection limit | 300           | 0.6          | 3             | 5             | 400           | 6             | 20            |
| 0%              | n.d.          | 251          | n.d.          | 14            | n.d.          | 16            | n.d.          |
| 0.10%           | n.d.          | 254          | n.d.          | 13            | n.d.          | 16            | n.d.          |
| 0.30%           | n.d.          | 261          | n.d.          | 13            | n.d.          | 17            | n.d.          |
| 1%              | n.d.          | 206          | n.d.          | 12            | n.d.          | 16            | n.d.          |
| 3%              | n.d.          | 218          | n.d.          | 12            | n.d.          | 17            | n.d.          |
| 10%             | n.d.          | 170          | n.d.          | 13            | n.d.          | 16            | n.d.          |

n.d.: lower than detection limit

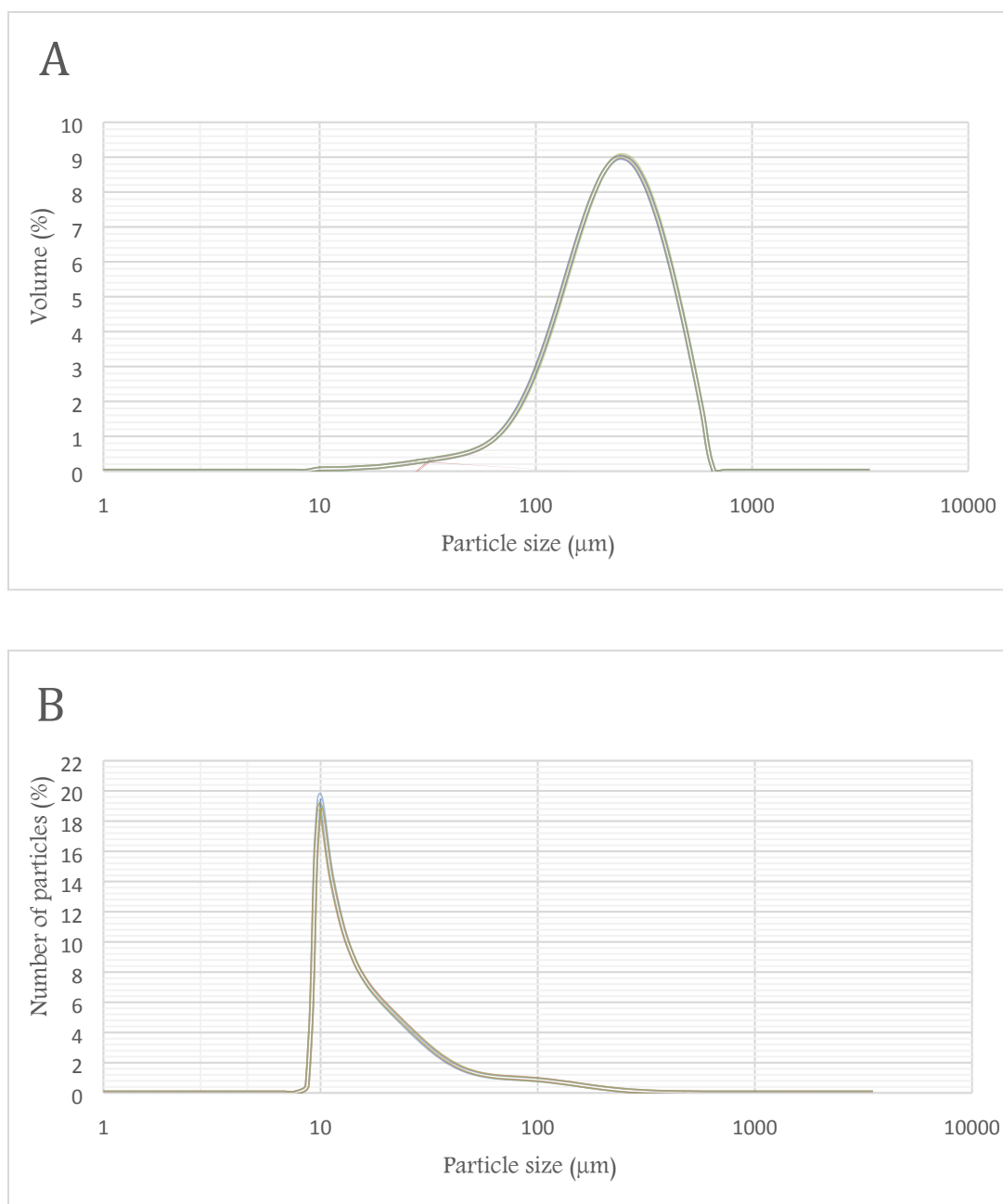
**Table S6. Concentration of PAHs (in mg/kg) at six car tire TP concentrations (0, 0.1, 0.3, 1.0, 3.0 and 10 %) in sediment DW.**

|                  | 0%   | 0.1%    | 0.3% | 1%    | 3%   | 10%  |
|------------------|------|---------|------|-------|------|------|
| Fluoreen         | 0.00 | 8.30*   | 0.79 | 0.40  | 0.00 | 0.36 |
| Fenanthrene      | 0.45 | 13.07*  | 0.39 | 0.48  | 0.41 | 0.47 |
| Anthracene       | 0.12 | 4.06*   | 0.09 | 0.13  | 0.10 | 0.09 |
| Fluoranthene     | 1.81 | 29.48*  | 1.38 | 2.04  | 1.53 | 1.48 |
| Pyrene           | 1.40 | 22.73*  | 1.24 | 1.65  | 1.67 | 2.26 |
| B(a)anthracene   | 0.78 | 12.82*  | 0.60 | 0.95  | 0.64 | 0.49 |
| Chrysene         | 0.74 | 14.96*  | 0.63 | 0.85  | 0.62 | 0.54 |
| B(e)pyrene       | 0.67 | 6.37*   | 0.47 | 0.74  | 0.58 | 0.54 |
| B(b)fluoranthene | 0.94 | 9.12*   | 0.65 | 1.03  | 0.75 | 0.58 |
| B(k)fluoranthene | 0.39 | 4.19*   | 0.27 | 0.44  | 0.32 | 0.23 |
| B(a)pyrene       | 0.75 | 7.83*   | 0.51 | 0.84  | 0.60 | 0.47 |
| Db(ah)anthracene | 0.52 | 4.51*   | 0.36 | 0.58  | 0.51 | 0.49 |
| B(ghi)perylene   | 0.09 | 0.95*   | 0.05 | 0.10  | 0.09 | 0.08 |
| Ind(123)pyrene   | 0.62 | 5.45*   | 0.37 | 0.61  | 0.49 | 0.34 |
| $\Sigma$ PAH     | 9.29 | 143.84* | 7.82 | 10.83 | 8.32 | 8.42 |

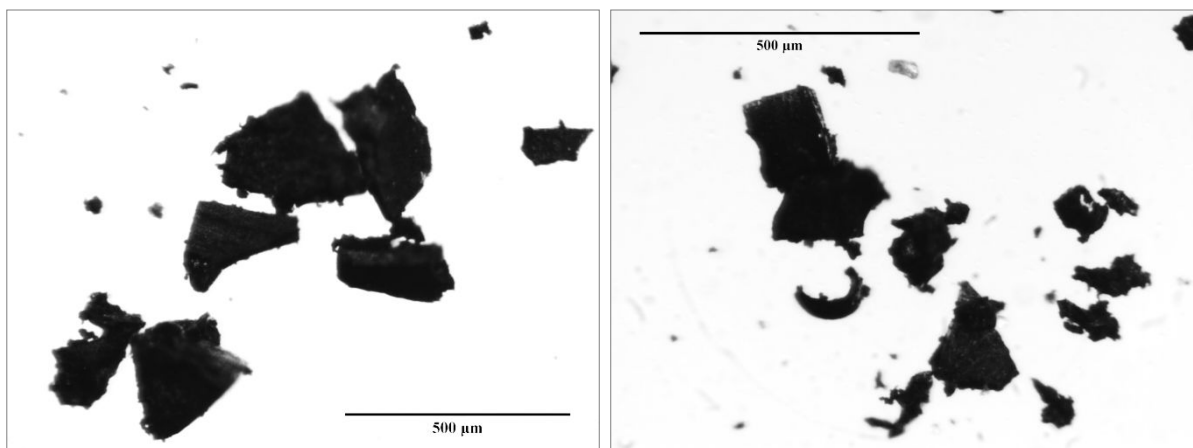
\* Outlier

**Table S7. Mean dry weight (in mg) and area (in mm<sup>2</sup>) of ten car tire tread particles per replicate (in quadruplicate) before and after their addition to H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>O for 24 hours.**

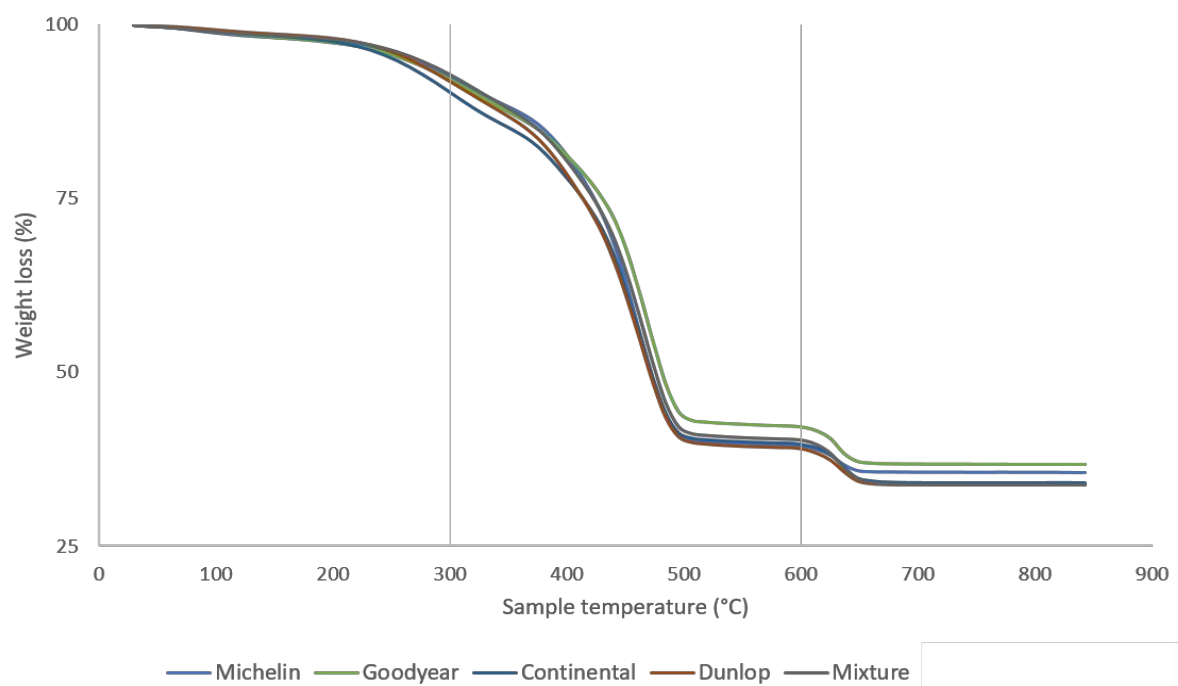
| Treatment                     | Replica | Dry Weight (mg) |              |                   | Area (mm <sup>2</sup> ) |              |                   |
|-------------------------------|---------|-----------------|--------------|-------------------|-------------------------|--------------|-------------------|
|                               |         | <i>Before</i>   | <i>After</i> | <i>Difference</i> | <i>Before</i>           | <i>After</i> | <i>Difference</i> |
| H <sub>2</sub> O <sub>2</sub> | 1       | 11,592          | 11,643       | -0,051            | 6,244                   | 6,343        | -0,099            |
|                               | 2       | 15,466          | 15,34        | 0,126             | 6,208                   | 6,109        | 0,098             |
|                               | 3       | 14,79           | 14,823       | -0,033            | 7,733                   | 7,755        | -0,022            |
|                               | 4       | 11,296          | 11,301       | -0,005            | 5,481                   | 5,571        | -0,089            |
| H <sub>2</sub> O              | 1       | 13,665          | 13,601       | 0,064             | 6,398                   | 6,374        | 0,024             |
|                               | 2       | 16,509          | 16,382       | 0,127             | 6,718                   | 6,706        | 0,012             |
|                               | 3       | 14,259          | 14,22        | 0,039             | 6,672                   | 6,683        | -0,011            |
|                               | 4       | 11,868          | 11,784       | 0,084             | 5,359                   | 5,322        | 0,037             |



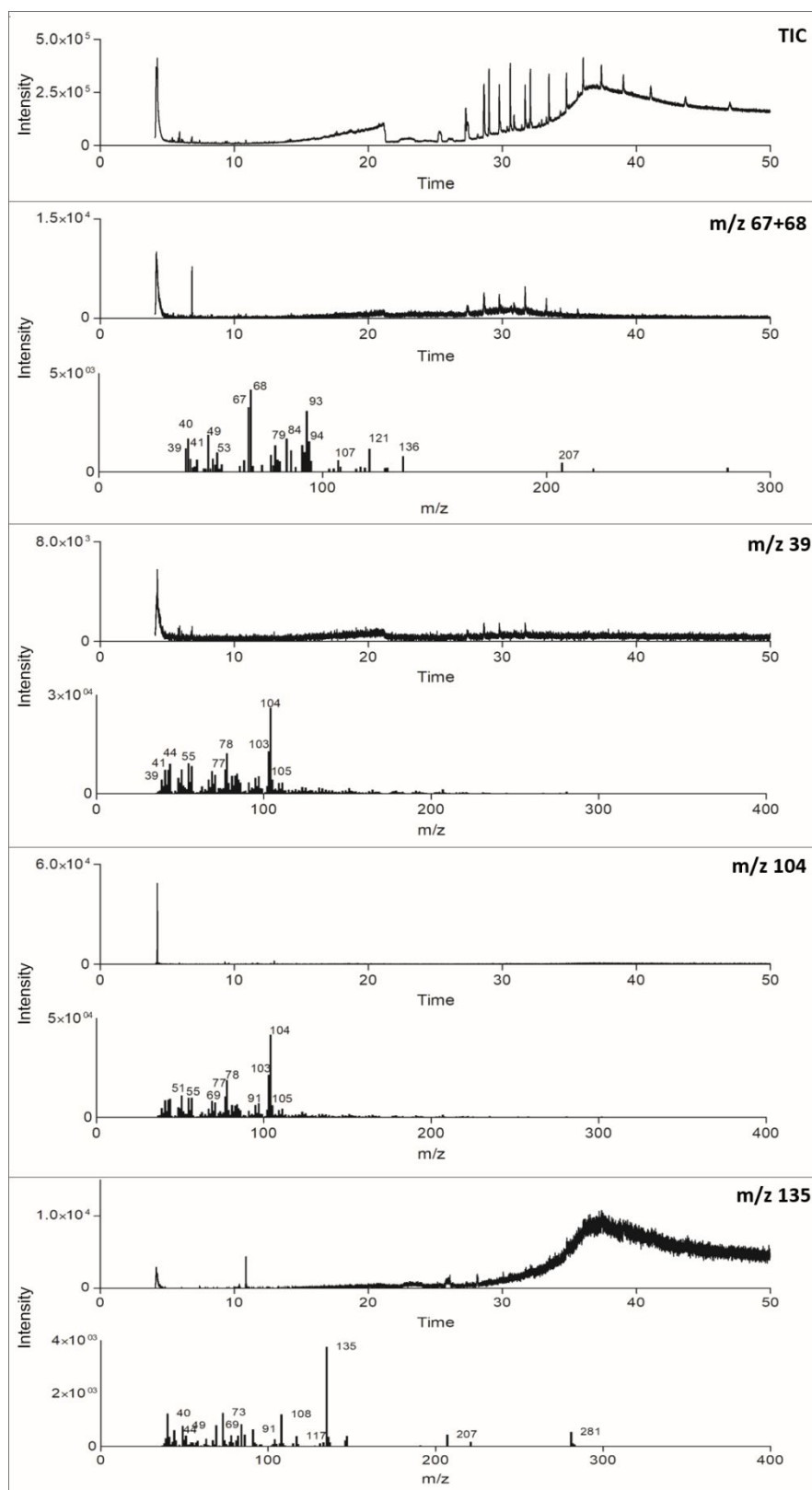
**Figure S1.** A) Particle size distribution of the car tire TP determined by volume of particles B) Particle size distribution of the car tire TP determined by the number of particles. Both graphs contain three repeated measurements.



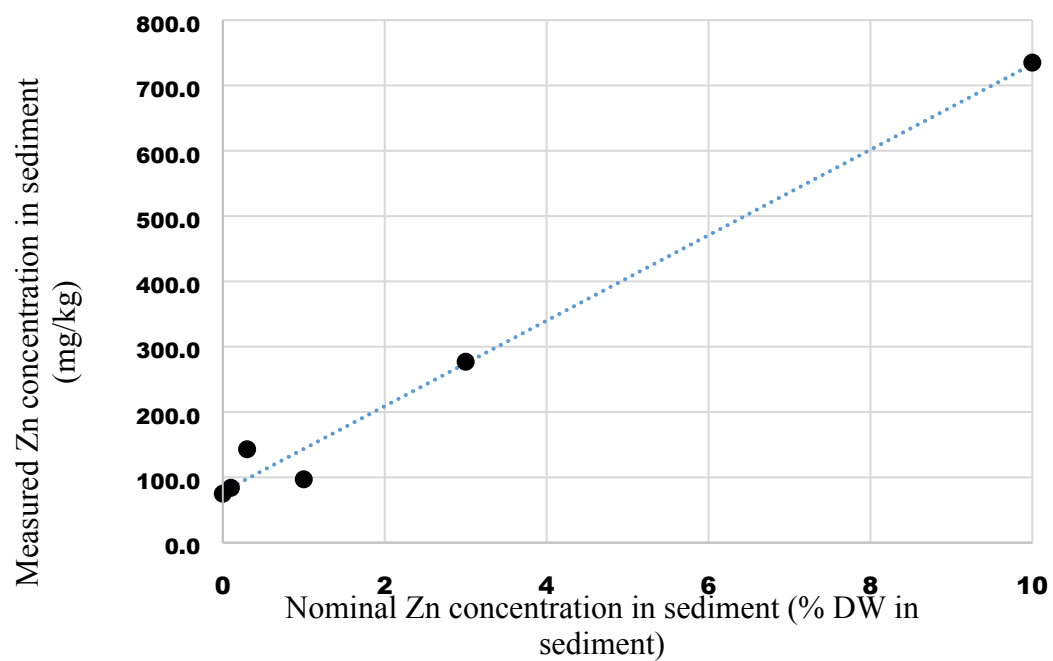
**Figure S2.** Pictures of the car tire TP mixture taken with a CMEX camera (Euromex, The Netherlands) under an Olympus SZX10 Stereomicroscope.



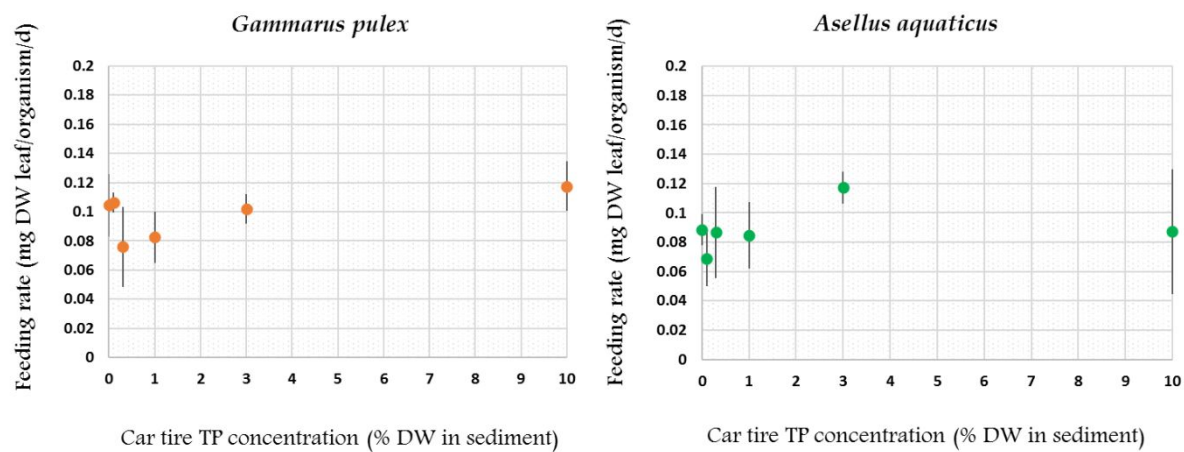
**Figure S3.** Weight loss (%) of individual car tire TP and their mixture using thermogravimetric analysis to distinguish between (i) volatile substances (that vaporize between 30- 300°C), (ii) the actual polymer (300- 600°C), (iii) carbon black (600- 850°C), and the residual (iv) inorganic fillers.



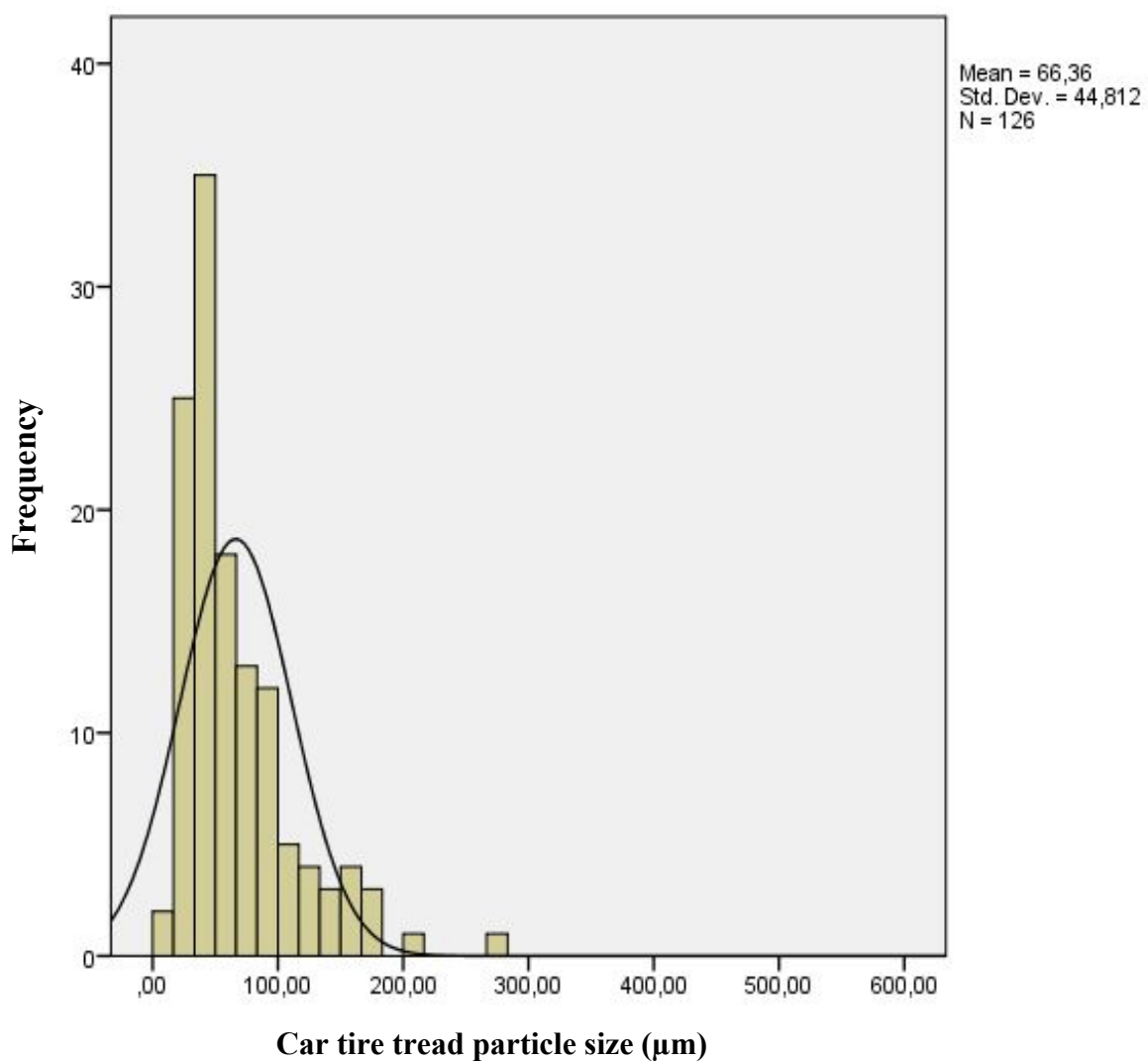
**Figure S4.** Pyrogram of the analyzed car tire TP represented by the total ion current (Total ion chromatogram, TIC, 1<sup>st</sup> Panel). Second – fifth panel: Ion chromatograms and mass spectra of identified polymer pyrolysates (see Table S3).



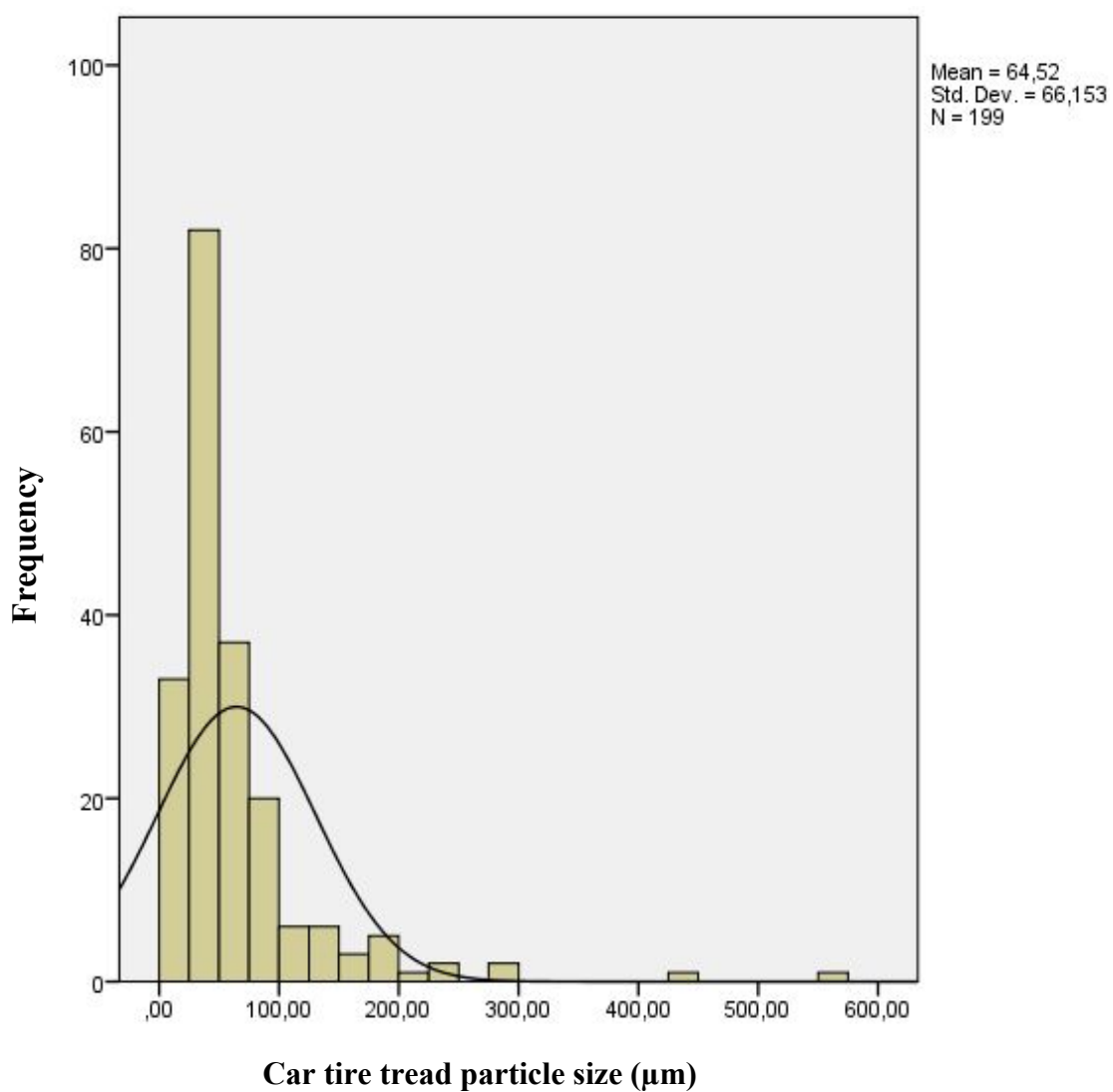
**Figure S5.** Nominal Zinc (Zn) concentration in sediment (X-Axis) against measured Zinc (Zn) concentration in TP-sediment mixtures (Y-Axis).



**Figure S6.** Feeding rate (mg DW leaf/organism/d) of *G. pulex* and *A. aquaticus* after 28 days of exposure to car tire TP at increasing concentrations in sediment. Error bars are mean  $\pm$  1 S.D. n=3.



**Figure S7.** Size frequency of the total number of car tire tread particles measured in the body of *G. pulex* at concentrations 3 and 10%.



**Figure S8.** Size frequency of the total number of car tire tread particles measured in faces of *G. pulex* at concentrations 3 and 10%.

## **PAHs analysis**

PAHs were extracted from the sediment-TP mixtures using accelerated solvent extraction (ASE) with a mixture of n-hexane and acetone (1:1, v/v) at 100 °C, heat time 5 min., static time 5 min., 2 cycles. The extractions were passed through a 10 % aluminium oxide column to remove polar components. The solution was concentrated on a modified Kuderna-Danish apparatus followed by evaporation to 1 mL under a gentle flow of nitrogen. Subsequently the sample was eluted with 30 mL of hexane over a column with 4 g of aluminium oxide (10% water), followed by evaporation to 1 mL under a gentle flow of nitrogen.. Samples were exchanged to acetonitrile, after which 2-methylchrysene was added as an internal standard. PAHs were analyzed on a Agilent 1100 High Performance Liquid Chromatograph (HPLC) equipped with 250 × 4.6 mm Vydac guard analytical reverse-phase C18 columns (201GD54T and 201TP54), with methanol/water as mobile phase. After each run, the columns were rinsed with acetonitrile. PAHs were detected on an HP 1100 multiwavelength fluorescence detector.

### Feeding rate<sup>2</sup>

$$FR = \frac{((L1 \times Cl) - L2)}{\left(\frac{Li1 + Li2}{2}\right) \times t}$$

where L1 is the initial and L2 the final dry weight of the *Populus sp.* disc (mg), Li1 and Li2 are the numbers of living organisms at the start and at the end of the experiment (Li1=11 individuals), Cl is the leaching-decomposition correction factor, calculated by dividing the initial dry weight by the final dry weight of the leaves in the control sample; and t is the incubation time (days).

### Supporting Information References

- (1) Gueissaz, L.; Massonnet, G. Study on the discrimination of tires using chemical profiles obtained by Py-GC/MS. *J. Anal. Appl. Pyrolysis* **2017**, *124*, 704–718.
- (2) Maltby, L.; Clayton, S. A.; Wood, R. M.; McLoughlin, N. Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: Robustness, responsiveness, and relevance. *Environ. Toxicol. Chem.* **2002**, *21*, 361–368.